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Engineered tumor-infiltrating macrophages as gene delivery vehicles for interferon-α activates immunity and inhibits breast cancer progression

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An immunosuppressive tumor microenvironment is a cancer hallmark and a major impediment to successful immunotherapy. We engineered hematopoietic progenitors to target expression of an interferon-α (IFNα) transgene specifically to their monocytic progeny, including tumor-infiltrating macrophages. Mice chimeric for these IFNα-expressing macrophages showed activation of innate and adaptive immune cells against breast cancer and inhibited disease progression.

Introduction

One area of cancer biology that shows promise in the identification of new therapeutic targets is the tumor microenvironment. By fostering tumor growth and progression, supporting angiogenesis and tissue remodelling, as well as by counteracting host immunity, the tumor microenvironment, including tumor-associated macrophages, plays an important role in modulating tumor progression and response to therapies. We recently identified and characterized a specific subpopulation of protumor macrophages characterized by the expression of the angiopoietin receptor TIE2.[1] These TIE2-expressing monocytes/macrophages (TEMs) are endowed with proangiogenic and immunosuppressive activities.[1-3] As a strategy aimed at reversing the immunosuppressive microenvironment, we exploited the tumor homing ability of TEMs and the upregulated expression of TIE2 in these cells to turn them into vehicles for interferon-α (IFNα) gene therapy. Type I interferons (IFNs) are pleiotropic cytokines involved in innate and adaptive immunity that have been shown to promote antitumor immune responses.[4] However, clinical use of IFNα has since declined because of the substantial toxicity associated with its systemic administration and the limited efficacy at the maximal tolerated doses, thus calling for safer and more effective delivery strategies. We engineered hematopoietic stem/progenitor cells (HSPCs) to express an *IFN*α transgene under the control of the *Tie2* promoter. Circulating TEMs express low levels of Tie2, which is, however, rapidly upregulated as they reach the tumor site. Whereas the tumor tropism of TEMs should allow effective delivery of IFNα to the tumor, the selective expression of the transgene should ensure low systemic exposure and, thus, toxicity. In a mouse model of breast cancer, our cell- and gene-based IFNα delivery strategy strongly inhibited primary tumors and lung metastasis by inducing the recruitment and activation of both innate and adaptive immune cells with no evident signs of toxicity.[5]

In an effort to bring our strategy to clinical testing, we recently developed a humanized vector expressing a human *IFN*α gene and studied the feasibility and safety of engineering human HSPC for its expression in their TEM progeny. Since the TIE2 promoter is also expressed in the most primitive HSPC and chronic exposure to IFNα can cause myelo-thrombocytopenia and long-term HSPC exhaustion,[6] we implemented our vector with transcriptional and posttranscriptional regulation mediated by the TIE2 enhancer/promoter and miR126/130a target sequences (miRT), respectively. By virtue of this bimodal strategy, we rendered our vector susceptible to the negative regulation of microRNA 126 and 130a, which are expressed in HSPC but not in mature blood cells. Using a GFP reporter cassette we demonstrated the selective expression of our new vector design in the myeloid progeny of lentiviral transduced human HSPC transplanted in immunodeficient NOD-SCID-IL2Rγ$^{-/-}$ (NSG) mice and confirmed effective microRNA-mediated suppression of transgene expression in the most primitive HSPC compartment. By monitoring NSG mice transplanted with HSPC transduced with TIE2-IFN-miRT

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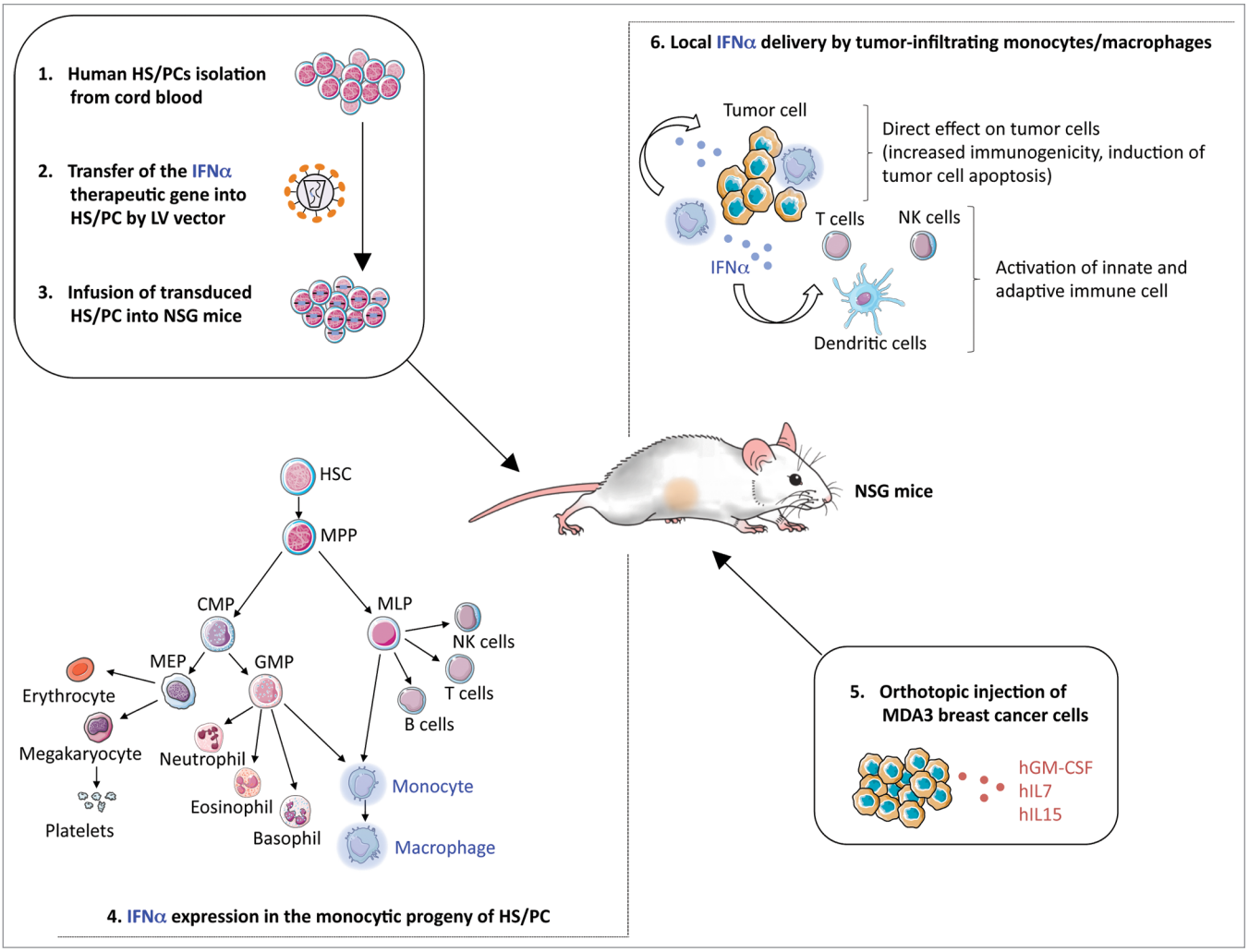

Figure 1. Experimental approach to assess the efficacy of our tumor-targeted IFNα gene-delivery strategy by tumor-infiltrating macrophages. Breakdown of steps involved in testing the efficacy of using TIE2-expressing macrophages to deliver interferon-α (IFNα) to the tumor to elicit local anti-cancer immune responses.[1-3] Cord blood derived human hematopoietic stem/progenitor cells (HS/PC) are transduced with the TIE2-IFN-mirT lentiviral vector (LV) and infused in sublethally irradiated immunodeficient NOD-SCID-IL2Rg$^{-/-}$ (NSG) mice.[4] Upon human hematopoietic cell reconstitution, only the monocytes/macrophages progeny derived from transduced HS/PC express the IFNα gene.[5] Reconstituted mice are orthotopically injected with MDA3 breast cancer cells engineered to release human granulocyte macrophage-colony stimulating factor (GM-CSF) and IL7, IL15 cytokines, which are required to foster the functional development of human T-, NK-, and antigen presenting cells.[6] Local delivery of IFNα by tumor-infiltrating monocytes/macrophages results in local activation of a type I IFN response and prompts the immune-mediated inhibition of tumor growth. Direct effects of IFNα on tumor cells (i.e., increased immunogenicity, induction of tumor cell apoptosis) and indirect effects on immune cells (i.e., enhanced NK and T cell cytotoxicity and enhanced cross-presentation ability of dendritic cells) may contribute to the induction of an antitumor response.

lentiviral construct (TIE2-IFN-miRT mice) we showed no evident abnormalities of reconstituted human hematopoiesis. More stringent safety studies performed in immune-competent mice using the mouse *Ifn*α gene also showed that our improved vector design could alleviate some of the effects of IFNα exposure on dormant HSPC (i.e., IFN-mediated cell cycle activation). Importantly, and in contrast to previous studies of increased exposure to IFNα (*6*), HSPC derived from TIE2-IFN-miRT mice did not exhaust upon transplantation into secondary recipients, thus indicating stringent containment of IFNα expression with our improved vector design. To demonstrate the efficacy of our strategy, we challenged human hematochimeric NSG mice with the human MDA-MB 231 breast tumor cell line modified to release human granulocyte macrophage-colony stimulating factor (GM-CSF) and IL7, IL15 cytokines required for the functional reconstitution of the human T-, natural killer (NK-), and antigen presenting cell compartments, which are otherwise limited in NSG mice. Strikingly, we found enhanced immune-mediated clearance of transplanted tumors in the TIE2-IFN-miRT group likely mediated by alloreactive human T cells developed in the mouse thymus—and thus tolerant to host tissues—as well as by the expanded NK cells. Such effective immune reaction was associated with local activation of a type I IFN response in the tumor, which in turn may have enhanced NK cell cytotoxicity and dendritic cell cross-priming

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ability resulting in more effective induction of T-cell responses (**Fig. 1**). When we applied our strategy to a syngeneic murine polyoma middle T (PyMT) breast cancer model and an experimental metastases model, we also observed enhanced generation of effector memory T cells and their recruitment to the neoplastic lesions that suppressed tumor growth and disease progression.

Our findings provide preclinical data supporting the feasibility and efficacy of engineering human hematopoiesis for tumor-targeted *IFN*α gene delivery by monocytes/macrophages. Recent studies have shown that tumor-infiltrating TEMs exert immunosuppressive and proangiogenic activity in human breast cancer and have implicated IFNα signaling to host immune cells as a key suppressor mechanism of bone metastases originating from primary breast tumors.[7,8] These results, together with our own, could revive interest in testing autologous HSPC transplantation in advanced breast cancer patients with the aim to effectively reprogram the tumor microenvironment and counteract the protumor activity of this endogenous population. Importantly as we showed therapeutic efficacy at low chimerism of transduced cells, we envisage genetic modification of only a fraction of HSPC to limit potential IFNα exposure and toxicity. Moreover our strategy could be tested in the treatment of hematopoietic malignancies, for which autologous transplantation is routinely used as standard treatment, and, for which IFNα has proven some anticancer efficacy in previous studies.[9] If demonstrated to be safe and effective in humans, our strategy could also be combined with other immunotherapies, as also suggested by recent works,[10] with the aim to create a synergy in eliciting powerful immune responses against established malignancies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. De Palma M, Venneri MA, Galli R, Sergi Sergi L, Politi LS, Sampaolesi M, Naldini L. Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. Cancer Cell 2005; 8:211-26; PMID:16169466; http://dx.doi.org/10.1016/j.ccr.2005.08.002
2. Coffelt SB, Chen YY, Muthana M, Welford AF, Tal AO, Scholz A, Plate KH, Reiss Y, Murdoch C, De Palma M, et al. Angiopoietin 2 stimulates TIE2-expressing monocytes to suppress T cell activation and to promote regulatory T cell expansion. J Immunol 2011; 186:4183-90; PMID:21368233; http://dx.doi.org/10.4049/jimmunol.1002802
3. Mazzieri R, Pucci F, Moi D, Zonari E, Ranghetti A, Berti A, Politi LS, Gentner B, Brown JL, Naldini L, et al. Targeting the ANG2/TIE2 axis inhibits tumor growth and metastasis by impairing angiogenesis and disabling rebounds of proangiogenic myeloid cells. Cancer Cell 2011; 19:512-26; PMID:21481792; http://dx.doi.org/10.1016/j.ccr.2011.02.005
4. Dunn GP, Bruce AT, Sheehan KC, Shankaran V, Uppaluri R, Bui JD, Diamond MS, Koebel CM, Arthur C, White JM, et al. A critical function for type I interferons in cancer immunoediting. Nat Immunol 2005; 6:722-9; PMID:15951814; http://dx.doi.org/10.1038/ni1213
5. De Palma M, Mazzieri R, Politi LS, Pucci F, Zonari E, Sitia G, Mazzoleni S, Moi D, Venneri MA, Indraccolo S, et al. Tumor-targeted interferon-alpha delivery by Tie2-expressing monocytes inhibits tumor growth and metastasis. Cancer Cell 2008; 14:299-311; PMID:18835032; http://dx.doi.org/10.1016/j.ccr.2008.09.004
6. Essers MA, Offner S, Blanco-Bose WE, Waibler Z, Kalinke U, Duchosal MA, Trumpp A. IFNalpha activates dormant haematopoietic stem cells in vivo. Nature 2009; 458:904-8; PMID:19212321; http://dx.doi.org/10.1038/nature07815
7. Ibberson M, Bron S, Guex N, Faes-van't Hull E, Ifticene-Treboux A, Henry L, Lehr HA, Delaloye JF, Coukos G, Xenarios I, et al. TIE-2 and VEGFR kinase activities drive immunosuppressive function of TIE-2-expressing monocytes in human breast tumors. Clin Cancer Res 2013; 19:3439-49; PMID:23649001; http://dx.doi.org/10.1158/1078-0432.CCR-12-3181
8. Bidwell BN, Slaney CY, Withana NP, Forster S, Cao Y, Loi S, Andrews D, Mikeska T, Mangan NE, Samarajiwa SA, et al. Silencing of Irf7 pathways in breast cancer cells promotes bone metastasis through immune escape. Nat Med 2012; 18:1224-31; PMID:22820642; http://dx.doi.org/10.1038/nm.2830
9. Trinchieri G. Type I interferon: friend or foe? J Exp Med 2010; 207:2053-63; PMID:20837696; http://dx.doi.org/10.1084/jem.20101664
10. Yang X, Zhang X, Fu ML, Weichselbaum RR, Gajewski TF, Guo Y, Fu YX. Targeting the tumor microenvironment with interferon-β bridges innate and adaptive immune responses. Cancer Cell 2014; 25:37-48; PMID:24434209; http://dx.doi.org/10.1016/j.ccr.2013.12.004

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